Cell-cycle regulation of a human histone H2b gene is mediated by the H2b subtype-specific consensus element

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Mammalian histone gene transcription is increased approximately fivefold during the transition from the G¹ phase to the S phase of the cell cycle. In this study, we present a detailed *in vivo* analysis of the human histone H2b promoter, which establishes that transcriptional regulation of this gene is mediated by a subtype-specific consensus element containing the core octanucleotide ATTTGCAT. Our results demonstrate that the activity of this sequence is specific for S phase. Comparative analysis of different replication variant mammalian histone gene promoters and our knowledge of the transcription factors interacting with the human histone H2b and H4 promoters allow us to conclude that coordinate regulation of histone gene transcription in higher eukaryotes is mediated by distinct factors. We propose a simple model for transcriptional regulation of mammalian histone gene expression, which incorporates both the distinct features of the individual histone gene promoters and the apparent functional equivalence of the specific sequence elements regulating transcription of each histone gene subtype.

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Once committed to divide, mammalian cells in culture execute a relatively well-defined program of progression toward mitosis. It is now well established that the transition from late G₁ phase into S phase is accompanied by the increased expression of many genes whose products are required for DNA synthesis or chromatin replication. Perhaps the most obvious example of this type of regulation was first described by Robbins and Borun (1967), when they discovered that histone protein synthesis occurs at significant rates only during the S phase in cultured mammalian cells. Subsequent studies have established that this reflects a similar increase in the concentration of histone mRNA during S phase, which is at least partly due to transcriptional induction of these genes as cells traverse the G₁/S phase boundary (Heintz et al. 1983; Sittman et al. 1983). These observations raise interesting questions concerning both the molecular mechanisms responsible for transcriptional induction of individual histone genes and the coordinate activation of transcription of the five different histone gene types during the transition from G₁ to S phase.

Several recent *in vitro* (Heintz and Roeder 1984; Hanly et al. 1985; Dailey et al. 1986; Sive et al. 1986;

Sive and Roeder 1986), and *in vivo* (Artishevsky et al. 1984, 1987; Capasso and Heintz 1985; Seiler-Tuyns and Paterson 1987), studies have demonstrated that mammalian histone gene expression is regulated by trans-acting transcription factors that act through promoter proximal DNA sequence elements. The detailed *in vitro* analyses of the human histone H4 and H2b promoters cited above have revealed the compact and complex nature of these promoters. Thus, maximal *in vitro* transcription of each of these promoters involves several distinct promoter proximal DNA sequence elements. However, in no case has it been established which of these promoter elements is responsible for the S phase induction of histone gene expression. It is unclear, therefore, whether a single set of proteins is responsible for the coordinate induction of mammalian histone gene transcription upon entry into S phase or whether different proteins can regulate the expression of the different histone gene subtypes.

In the case of the histone H2b promoter (Sive et al. 1986), analysis of a comprehensive series of deletion, linker scanning, and point mutations demonstrated that the TATA box, a subtype-specific consensus element, a CCAAT homology, and a series of small direct repeats were all essential for efficient transcription. In this study, we have assayed the contribution of the histone H2b promoter elements to transcription during the cell

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cycle *in vivo*. We find that the histone H2b subtype-specific consensus element, containing the core octanucleotide ATTTGCAT, mediates transcriptional induction at the G₁/S phase boundary and that the remaining promoter proximal DNA sequence elements are constitutively active during this transition. Because this element is specific for histone H2b transcription (Fletcher et al. 1987), it is apparent that the transcription activation of the different mammalian histone gene subtypes upon entry into S phase is controlled by distinct proteins. These data suggest a model for mammalian histone gene expression that is quite different from that proposed recently for yeast histone gene expression (Osley et al. 1986) and that may be relevant to the coordinate induction of other S phase-regulated nonhistone genes.

Results

DNA constructions and S1-mapping probes

To assay the effects of specific mutations on the utilization of the human histone H2b promoter *in vivo*, we have employed fusion genes in which the H2b promoter DNA is coupled to the bacterial chloramphenicol acetyltransferase (CAT) gene (Gorman et al. 1982). As shown in Figure 1, the specific constructs we have used in these studies are a subset of those described by Sive et al. (1986) and were chosen to specifically assay the contributions of each H2b promoter element. Because the mRNA produced from each of the recombinant H2b constructs is identical and because our *in vitro* analysis demonstrated that these alterations in the promoter directly affect the rate of transcription (Sive et al. 1986), we are confident that the accumulation of the fusion mRNA is a direct consequence of transcription. The internal control fusion genes we have cotransfected as standards for quantitation of the relative phenotypes for each of the test genes contain either the SV40 early promoter (pSV2CAT, Gorman et al. 1982) or sequences be-

tween -38 and +60 of a human histone H1 promoter (F. LaBella and N. Heintz, unpubl.). In each analysis presented below, nuclease S1 mapping was used to measure the amount of transcript produced from both the test and control genes. As diagrammed, the utilization of the CAT gene body to assay the activities of both the test and control promoters allowed us to use a single probe to detect both of the transcripts in a single reaction. Thus, a DNA probe 5'-end labeled at the *Eco*RI site within the H2b/CAT coding region will fully protect the H2b/CAT fusion transcript (290 bp) but will protect either of the control genes only through the shared CAT sequences (~250 bp).

Expression of the H2b promoter mutants in synchronized 293 cells

Our preliminary analysis of the wild-type histone H2b/CAT gene fusion during transient expression in a variety of human cultured cell lines established that the adenovirus E1A-transformed human embryonic kidney cell line 293 expressed this gene at significantly elevated levels. To ensure maximum sensitivity for the comparison of the various altered H2b promoters, our initial studies have been completed in this cell type. The protocol we have employed to assess regulation during the G₁ to S phase transition is detailed in Methods and involves arrest of the transfected cell population in S phase by a single block with aphidicolin. Upon release from the drug blockade, the rate of DNA synthesis increases approximately 10-fold (Heintz et al. 1983; data not shown). As shown in Figure 2B, this increase in DNA synthesis is accompanied by a parallel increase in the steady-state concentration of histone H4 mRNA.

It is immediately apparent from inspection of the S1 nuclease mapping data presented in Figure 2A that the protocol we have chosen for this study can provide information concerning both constitutive and regulated expression of the H2b gene. Although these data graphi-

Figure 1. Diagram of H2b/CAT fusion genes and S1 mapping strategy. Sequences and position of the upstream elements of the H2b promoter are shown with the relevant mutants used in this study. Point mutations that inactivate the hexamer (hex⁻) and octamer (oct⁻) are indicated above these elements with arrows pointing to the substituted nucleotides. The transcription start site is indicated as +1; the CAT coding region is depicted by an open box, the *Eco*RI site positioned at +290 was used to prepare a 5' end-labeled S1 probe. The expected S1-nuclease-protected transcripts in a typical cotransfection experiment are indicated: RNA transcribed by H2b/CAT fusion gene is expected to yield a hybrid of ~290 nucleotides, whereas the one derived from the internal control promoter (either SV40 or H1) should be protected only through the CAT coding sequences, resulting in protection of ~250 nucleotides.

Figure 2. Expression of H2b promoter mutants in synchronized 293 cells. Cells were cotransfected with H2b/CAT and pSV2CAT genes and synchronized as described in the text. Total RNA was extracted at ~40 hr after DNA transfection, and 5 μ g were S1 mapped using the probe shown in Fig. 1. Names of the transfected mutants are indicated above each pair of lanes; (0, 3). Hours after release into S phase when RNA was extracted. (A) The large and small arrows on the side of the autoradiogram point to the H2b/CAT and the SV2CAT protected bands, respectively. (B) In parallel, 2 μ g of the same RNA samples was hybridized to 5'-end-labeled NarI-pHu4a (H4) probe (●).

cally reveal several interesting features of histone H2b transcriptional regulation in vivo, these points are reinforced by quantitation of similar data derived from several independent transfections (Table 1). The first point to be made from these data is the identification of the H2b cell-cycle regulatory element. Mutants that delete or inactivate any sequence upstream from the H2b subtype-specific consensus sequence retain the ability to be transcriptionally activated upon entry into S phase. Thus, deletion of sequences distal to position -77, including both the direct repeats and the CCAAT box, does not affect the ability of these constructs to be induced approximately fourfold upon release from the DNA synthesis block. The same level of induction is retained in the hex⁻ mutant, in which the GACTTC consensus element at position -70 has been inactivated by point mutations in the context of the wild-type H2b promoter. Therefore, none of these promoter distal elements is required for cell-cycle regulation in vivo. In

contrast, mutation of the core octanucleotide sequence of the H2b subtype-specific consensus element in the context of the wild-type promoter eliminates the transcriptional induction of the H2b promoter upon entry into S phase. These results identify this subtype-specific sequence as the critical element for cell-cycle regulation of the human histone H2b gene in vivo.

The second point to be made from these data concerns the contribution of the various promoter elements that were identified in vitro (Sive et al. 1986) to both the basal and S phase level of expression of the H2b gene in vivo. With the possible exception of the GACTTC element, each of the sequences that contributes to efficient expression in vitro is also required for maximal transcription in vivo. Thus, deletion of the distal H2b promoter sequences, including the direct repeats and the CCAAT box, results in significant reduction in both basal and induced transcription in vivo. In the most extreme case, deletion of all sequences upstream of posi-

Table 1. Accumulation of H2b/CAT fusion mRNAs following release into S phase in vivo

Mutant	293 cells			Percent in vivo		Percent in vitro	HeLa cells		
	T = 0	T = 3	T = 3/T = 0	T = 0	T = 3		T = 0	T = 3	T = 3/T = 0
-147 (4)	0.95	3.71	3.90	100	100	100	ND	ND	ND
-118 (3)	1.20	5.13	4.28	126	100	97	(4) 1.00	2.70	2.70
-100 (3)	0.55	2.47	4.50	57	66	48	ND	ND	ND
-82 (3)	0.18	0.99	5.50	19	26	33	ND	ND	ND
-77 (3)	0.05	0.21	4.16	5	6	25	ND	ND	ND
Hex ⁻ (2)	1.15	4.16	3.60	118	112	51	(3) 0.90	2.25	2.50
Oct ⁻ (5)	1.21	1.17	0.97	127	31	15	(4) 0.85	0.95	1.10

Summary of H2b promoter analysis in 293 and HeLa cells. Values given are the means of at least two independent transfection experiments (actual number given in parentheses), presented either as absolute value or as percentage of wild-type transcription. In all cases, values were normalized to mRNA produced by cotransfected pSV2CAT plasmid. Quantitation was performed densitometrically, and at least two different exposures of each autoradiogram were scanned to ensure linearity. For comparison, values derived from in vitro analysis of H2b promoter mutants are included. (ND) Not done.

tion -77 results in transcription at only 5% of the wild-type level, either prior to release from the DNA synthesis inhibitor or during S phase. These data therefore demonstrate that the H2b promoter distal sequence elements are extremely important for efficient transcription *in vivo* but that they act constitutively during the cell cycle. The fact that the hex^- mutation has no effect on transcription in this assay suggests either that the minor effect we have previously reported is an artifact of the *in vitro* system or that the factor(s) interacting with this conserved sequence is either limiting or not essential *in vivo*.

One of the most interesting facts revealed in this analysis is that the effect of the point mutations in the H2b octamer sequence is specific to S phase. Thus, transcription of this template DNA *in vivo* occurs at the wild-type level prior to release into S phase. There is simply no increase in utilization of this mutant promoter as the cells begin DNA synthesis. The fact that this mutation has no effect on H2b basal transcription indicates that the constitutive H2b promoter elements do not require the subtype-specific DNA consensus element for their activity. In this case, therefore, there is no evidence supporting a cooperative interaction between the proteins interacting with the closely opposed constitutive and regulatory sites in the histone H2b promoter.

A final point to be made from these data is that this type of transfection experiment has failed to reveal any significant negative component for repression of histone gene transcription prior to release into S phase. Thus, none of the mutants tested displays a significantly higher basal level of transcription than the wild-type H2b promoter. Furthermore, the magnitude of the transcriptional response is consistent with prior measurements (Heintz et al. 1983; Sittman et al. 1983) of the transcriptional induction of the genomic histone gene population, suggesting that the methodology is accurately reproducing this regulatory event.

Expression of histone H2b promoter constructs in synchronized HeLa cells

To demonstrate that the regulation of the H2b promoter in 293 cells did not result from the action of the adenovirus E1A gene product, we have analyzed the expression of several of the H2b fusion genes in synchronized HeLa cells. Our initial experiments in HeLa cells again utilized the pSV2CAT construct as an internal control and resulted in very high levels of transcription from the control template but essentially no transcription from the H2b fusion genes (F. LaBella, unpubl.). Because the SV40 enhancer present in pSV2CAT contains the octamer ATTTGCAT, which forms the core of the H2b subtype-specific element, it appeared that this effect might be explained by competition for the protein binding to this sequence. The fact that the E1A gene product can suppress the activity of the SV40 enhancer (Velcich and Ziff 1985) could provide a reasonable explanation for the different competitive strength of this sequence in 293 cells versus HeLa cells.

To circumvent this problem, and as an initial indication that this hypothesis is correct, we have repeated the transfection experiments in HeLa cells using as an internal control a human histone H1/CAT fusion gene in which the promoter has been truncated immediately upstream from the TATA box. As shown in Figure 3, in this case, the H2b promoter functions and displays the same regulatory characteristics that we have described above. Thus, the constructs carrying an intact H2b subtype-specific element are transcriptionally induced upon entry into S phase, whereas the fusion gene carrying the point mutations in the H2b octamer sequence is constitutively expressed. It is apparent from these data, and from several repetitions of this experiment, that utilization of the H2b promoter elements is essentially identical in HeLa and 293 cells.

The only differences that we have routinely encountered in the HeLa cell assays are a greater variation in

Figure 3. Expression of H2b promoter mutants in synchronized HeLa cells. Cells were cotransfected with H2b/CAT and -39 H1/CAT fusion genes and synchronized as described in the text. 20 μg of total RNA was S1 mapped in each experiment. The mutants analyzed and the hours after release into S phase when RNA was prepared are indicated above the lanes. The large arrow points to H2b/CAT mRNA; the small one to the control H1/CAT mRNA. The larger bands in these experiments (*) are due to transcription initiated within vector sequences that are discontinuous with the S1 probe at the position of the deletion (see text). (A, B) Independent transfection experiments.

the efficiency of transfection and a less pronounced induction of H2b transcription upon entry into S phase (see Table 1.) For example, the absolute levels of expression of the various H2b/CAT fusion genes are clearly quite different in the two experiments shown in Figure 3 (compare panels A and B), although the behavior of these genes upon entry into S phase is consistent between experiments. Furthermore, the changes in the levels of both the internal control H1/CAT fusion mRNA and the adventitious higher molecular weight transcript in the -77 lanes of Figure 3, panel A, result from this variability in transfection efficiency. This conclusion is based on the facts that the concentration of the internal control and plasmid-promoted transcripts do not increase upon entry into S phase in the other samples analyzed in these experiments (e.g., Fig. 3, panel B, -118 and -77 lanes). The plasmid-promoted transcripts are evident only in samples in which the S1 probe being employed is prepared from constructs containing more 5'-flanking DNA than that present in the construct analyzed. The probe employed in Fig. 3, panel A, was prepared from the -118 H2b/CAT fusion plasmid and, therefore, resulted in additional bands only in the -77 construct. The probe used in Fig. 3, panel B, was derived from the -147 H2b/CAT plasmid, resulting in additional bands in both the -118 and -77 constructs. We have not mapped the actual initiation site of these mRNAs within the vector DNA. Finally, the expression of the H2b fusion gene deleted to position -77 is so depressed that accurate quantitation of the activity of this promoter is not possible. It is evident, however, from visual inspection of several separate experiments that the level of expression of this gene is elevated upon entry into S phase (data not shown). We believe that these problems reflect technical difficulties with the HeLa cell assays and that they are not important biologically. It is probable, therefore, that the H2b subtype-specific element mediates H2b transcriptional regulation in all dividing cells.

Kinetics of induction of H2b transcription following release from the aphidicolin block

The results we have presented above indicate that although histone gene transcription is induced only approximately fivefold upon release into S phase, this increased transcription results from utilization of a sequence element that is essentially inactive in arrested cells. Thus, a relatively modest increase in the rate of transcription reflects a qualitative change in the activity of the subtype-specific sequence element. Previous *in vivo* measurements have established that full transcriptional induction of histone gene expression is evident within 10 min after reversal of a DNA synthesis block (Graves and Marzluff 1984). To further assess the utility of the transient expression assays for the analysis of histone gene transcriptional control during the cell cycle, we have compared the kinetics of induction of the transfected histone H2b/CAT fusion mRNA with that of the

corresponding endogenous wild-type histone H2b mRNA.

As shown in Figure 4, very little accumulation of either mRNA is observed during the first 30 min following removal of the DNA synthesis inhibitor. This probably reflects the time necessary for the cells to recover from the manipulations required for removing the aphidicolin, as the rate of DNA synthesis does not increase as rapidly in these cells as it does in similarly synchronized cultures of cells growing in suspension (F. LaBella and N. Heintz, unpubl.). Both the endogenous histone H2b mRNA and the H2b/CAT fusion mRNA begin to accumulate between 30 min and 1 hr postrelease. It appears, therefore, that the increased copy number of the episomal DNA in transfected cells does not result in an alteration in the timing of transcriptional induction upon entry into S phase. However, the steady-state levels of the H2b fusion transcripts plateau at approximately 1 hr postrelease, whereas the endogenous H2b transcripts continue to accumulate until at least 4 hr postrelease. It is probable that this difference reflects the lack of post-transcriptional control of the fusion mRNA and a significant contribution of posttranscriptional regulation to the accumulation of the wild-type H2b transcripts during S phase.

Discussion

In this study we have analyzed the effects of a variety of promoter mutations on the induction of histone H2b

Figure 4. Kinetics of induction of wild-type H2b in synchronized HeLa cells. Cells were cotransfected with -118 H2b/CAT Δ and -39 H1/CAT $\circ$ and synchronized as described in the text. At 0, 0.5, 1, 2, 4, and 8 hr after release into S phase, total RNA was prepared, and the levels of the CAT fusion mRNAs and the corresponding endogenous histone H2b mRNA $\blacksquare$ were determined by S1 mapping as in Fig. 2. Values were obtained by excising the appropriate radioactive bands from the gel and counting in a scintillation counter.

transcription following release from an aphidicolin block at the G₁/S phase boundary. We strongly believe that this response accurately reflects the transcriptional induction of histone gene expression upon entry into S phase in untreated cell populations because a similar magnitude of increase in transcription of the endogenous histone genes and the close temporal coupling to the onset DNA synthesis have been observed in cells synchronized by a variety of different treatments (Heintz et al. 1983; Sittman et al. 1983; Graves and Marzluff 1984) and upon entry into S after serum stimulation (DeLisle et al. 1983; Greenberg and Ziff 1984). It is possible, however, that the regulation of histone gene expression during the transition from quiescence to growth (the G₀ to S phase transition) could involve additional controls not evident in the synchronized cell system utilized for these experiments.

The results we have presented in this study suggest a rather simple model for the regulation of H2b transcription during the G₁ to S phase transition. As shown in Figure 5, constitutive transcription is mediated by two separable promoter domains: the core promoter, which has been shown in several cases to be responsible for accurate transcription initiation; and the distal activating domain, which increases the efficiency of transcription approximately 20-fold. These domains provide sites for assembly of a complex that can mediate transcription initiation at a significant rate independent of position in the cell cycle. The regulatory domain of this promoter, the H2b subtype-specific consensus sequence, is responsible for the induction of transcription during S phase and can function in the absence of the distal activating domain. In the simplest case, therefore, transcriptional regulation of histone H2b expression during the cell cycle may involve activation of a single transcription factor whose activity is effected through binding to the H2b subtype-specific consensus element. In a separate study (Fletcher et al. 1987), we present the purification of a 90-kD transcription factor that functions through the H2b regulatory sequence and is presumably the factor responsible for transcriptional induction upon entry into S phase.

The results presented in this study support our previous *in vitro* studies of histone H4 transcriptional regulation *in vitro* (Heintz and Roeder 1984; Hanly et al. 1985) and extend those studies to definitively establish that mammalian histone gene regulation *in vivo* is mediated by promoter proximal *cis*-acting DNA sequences. The identification of the H2b subtype-specific element as the transcriptional regulatory domain of this promoter allows several conclusions to be drawn concerning the transcriptional regulation of mammalian histone gene expression. Perhaps the most interesting conclusion is that coordinate regulation of the different histone gene subtypes is mediated by different transcription factors. The 90-kD transcription factor (Fletcher et al. 1987) interacting with the H2b regulatory domain does not interact with the histone H4 promoter. Nor do the H4 transcription factors interact with the H2b promoter (Dailey et al. 1986). The coordinate activation of

Figure 5. Model showing general organization of a mammalian histone gene promoter. Sequence elements shown are those specifically analyzed in the H2b promoter. The domain structure proposed emphasizes the functional equivalence of these domains in the promoters controlling transcription of the various histone gene subtypes.

mammalian histone gene transcription at the G₁/S phase boundary is, therefore, achieved through the activation of distinct and functionally equivalent transcription factors. Thus, the mechanism for activation of the histone gene transcription factors is pleiotropic and could be important for the regulated expression of other S phase-induced genes.

A second interesting issue raised by these studies concerns the subtype-specific consensus elements present in mammalian histone genes (Perry et al. 1985; Wells 1986) and their relationship to transcriptional regulation. In the case of the histone H4, H2b, and H1 genes, the subtype-specific sequences are very highly conserved in sequence, position, and orientation. We have established that the H2b subtype-specific element is responsible for cell cycle regulation and suggest that the equivalent elements in the H4 and H1 promoters may function in an analogous manner. In this case, the precise positioning of the regulatory site in the histone promoter may have important mechanistic implications. Perhaps the close apposition of the regulatory proteins and the general transcription initiation factors is essential for the regulatory role of these proteins in the initiation of transcription during the cell cycle. It is quite possible, for instance, that the activation of such factors as cells enter S phase could involve a functional modification that is active only if properly presented to the initiation complex. The lack of very highly conserved subtype-specific consensus elements at this position in the histone H2A and H3 genes suggests that this model may not apply to all histone gene subtypes. In fact, a very recent study of the mouse histone H3 promoter (Artishevsky et al. 1987) indicates that regulation of this gene may depend solely upon sequences approximately 150 bp upstream from the transcription initiation site.

The experimental approaches we have employed to assess the regulation of the H2b gene *in vivo* suggest two additional points. First, the fact that transcriptional regulation occurs in the transient expression assays indicates that chromosomal position is not important for regulation of the mammalian histone gene family. Second, it is very significant that the mutation in the H2b regulatory site has no effect on transcription in cells

arrested at the G₁/S phase boundary but prevents the induction of H2b transcription upon release from the DNA synthesis block (see Table 1). Because cells arrested in this way have completed the G₁ phase, it is apparent that the activation of the H2b regulatory factor is coincident with or dependent upon DNA synthesis. This is consistent with previous data concerning the dynamic control of histone gene regulation in mammalian cells (Heintz et al. 1983; Artishevsky et al. 1984; Graves et al. 1984). Although we believe that the regulatory event we have analyzed in this paper is sufficient to account for the transcriptional control of histone gene expression in actively growing cells, other modes of histone gene transcriptional regulation may be important in cells that have exited the cell cycle. It remains possible, for example, that these genes are subject to additional negative control mechanisms in quiescent cells.

Finally, it is important to note that the properties we have described for mammalian histone gene regulation during the cell cycle are quite different from those defined in the analysis of yeast histone gene transcriptional regulation. Studies employing conditional mutants that arrest cells in late G₁ phase suggest that in both budding (Hereford et al. 1982) and fission (Matsumoto et al. 1987) yeasts, histone gene transcription is activated prior to the G₁/S phase boundary. In contrast, the fact that mutations in the regulatory domain of the human histone H2b promoter have no effect on transcription until release from the DNA synthesis inhibitor indicates that transcriptional induction of mammalian histone gene expression is not fully activated until cells enter S phase. Furthermore, it is probable that in both yeasts, the coordinate activation of the different histone gene subtypes is accomplished through a single regulatory mechanism (Matsumoto and Yanagida 1985; Osley et al. 1986). As discussed above, it is quite clear that coordinate transcription of the human histone H4 and H2b genes is achieved using distinct transcription factors. In the case of higher eukaryotes, therefore, it is probable that the pleiotropic step responsible for coordinating the transcription of the different histone gene subtypes results in the functional modification of these factors as cells enter S phase. Finally, it is clear from the recent work of Osley et al. (1986) that periodic transcription of *Saccharomyces cerevisiae* histone genes during the cell cycle involves a negatively acting upstream sequence element. Removal of this element results in constitutive expression of the histone genes. The transfection experiments we have presented in this study establish that such a negatively acting element is not required for transcriptional regulation of the human histone H2b gene in actively cycling human cells. Such an element may, however, function in quiescent cells to further limit the activity of the mammalian histone genes.

Methods

Cells, transfection, and synchronization

HeLa and 293 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. Cells

were plated at 7×10^5 /100-mm dish, and 24 hr later transfected with 20 μ g of plasmid DNA (10 μ g of test gene + 10 μ g of control gene) by the calcium phosphate coprecipitation technique (Graham and Van der Eb 1973). The precipitate was left on the cells for 24 hr, after which the medium was replaced with fresh medium containing 5 μ g/ml aphidicolin (Pedrali-Noy et al. 1980). Synchronization was achieved by exposing the cells to the drug for 14–16 hr, followed by release into S phase by several washes in PBS and media replacement. DNA synthesis was routinely monitored by [³H]dCTP incorporation (Nilsen and Baglioni 1979).

Construction of fusion genes

A subset of the 5'-deletion and point mutants in the H2b promoter, described by Sive et al. (1986), was employed in this study: -147, -118, -100, -82, -77, OM-F (Hex⁻) and OM-A (Oct⁻). These promoters were fused to the bacterial CAT gene by cloning the *Hind*III-*Alu*I fragment of H2b into a *Hind*III blunted *Sal*I double-cut pUC18 vector in which the CAT coding region had been previously inserted into the *Bam*HI site (named ppCAT vector). The *Alu* site is situated 28 bp downstream of the H2b CAP site, and the first AUG to be utilized is the bacterial translation start.

RNA analysis

Total RNA was extracted from HeLa or 293 cells at various times after release into S phase: 0, 0.5, 1, 2, 4, and 8 hr for the kinetics of induction experiment and 0 and 3 hr for all the others. 5–20 μ g of total RNA was S1 mapped according to established procedures (Berk and Sharp 1978). DNA-RNA hybridization was performed at either 45°C (for CAT fusion gene analysis) or 48°C (for endogenous H2b and H4 genes). S1 probes were prepared as follows: (1) -118 H2b/CAT plasmid cut at the *Eco*RI site was 5'-end labeled to map H2b/CAT and control CAT mRNAs; (2) pHu4a cut at the *Nar*I site was 5'-end labeled to map endogenous H4 mRNA (Capasso et al. 1987); (3) pHh4a cut at the *Eco*RI site was 3'-end labeled to map endogenous H2b mRNA (Zhong et al. 1983).

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